OBSERVATIONS OF SOME NEMATODES FROM KANGAROO ISLAND, SOUTH AUSTRALIA, INCLUDING THE DESCRIPTION OF A NEW SPECIES, HEMICYCLIOPHORA FLUVIALIS (TYLENCHIDA: HEMICYCLIOPHORIDAE), FROM ROCKY RIVER

by Alan E. Bird

Summary

BIRO, A. F. (1999) Observations of some nematodes from Kangaroo Island. South Australia, including the description of a new species, *Hemicycliophora Illuvialis* (Tylenchida: Hemicycliophoridae), from Rocky River. *Trans. R. Soc. S. Aust.* **123**(4), 121-131, 30 November, 1999.

A new species of Hemicycliophora De Man, 1921 is described from Rocky River which runs through the Flinders Chase National Park on Kangaroo Island.

The morphology of the new species. Hemicycliophora fluvialis, is compared with that of four mainland South Australian species of this genus. It resembles H. chartestom Reay, 1984 more closely than the after South Australian species. A population of hatobrilus heptapapillatus (laubert & Heyns, 1979) Isololikhin, 1981 is also described from Rocky River and is compared with populations of this species from mainland Australia and South Africa. A population of Hemicriconemoides minur Brzeski & Reay, 1982 collected from soil adjacent to Rocky River is compared with specimens from Kuitpo Forest, 30 km south of Adelaide, Relationships between these Kangaroo Island nematodes and their close relatives on the South Australian mainland are discussed.

Key Words: Hemicycliophora fluvialis sp. 160v., Eutobrilus heptapapillatus, Hemicyconemoides minor, Rocky Rîver, Kangarou Island, nematodes, morphology, measurements.

Introduction

Rocky River is one of the more pristine rivers or streams on Kangaroo Island running, as it does, through Flinders Chase National Park throughout its length and thus being free from pollution from farmed lands and human habitation. Its nematode microfauna has not been studied or compared with mainland species. Kangaroo Island is thought to have been separated from the mainland for about 9,500 years (Lampert 1979) and some divergence from the mainland populations might be expected.

In this paper the ionic composition of the water from several of the island's rivers that run through farm lands is compared with that from Rocky River, Measurements of some free-living and plant parasific nematodes are made and compared with related mainland species. These relationships are discussed and a new species is described.

Materials and Methods

Sites

Soil and water samples were collected from the Rocky River site (1) (35° 57° S, 136° 42° E) on two occasions, firstly on 3 June 1993 and secondly, four years later, on 5 October 1997. On the first occasion samples were collected from other rivers on Kangaroo Island (Fig. 1) for comparison. These sites, in order of increasing salimity, were (2) Stunsail

Boom River, collected on the seaward side of the bridge across the river on the South Coast Rd. (3) Harriet River, collected on the seaward side of the bridge across the river on the South Coast Rd. (4) Eleanor River, collected close to the bridge across the river on the South Coast Rd. (5) Chapman River, collected on the landward side of Willoughby Rd and (6) Cygnet River, collected about 50 m up stream of the bridge at the township.

Collection and processing of samples

Water samples were filtered through a 0.2 µm membrane filter and stored in sterile serew-capped bottles prior to analyses of major soluble ions as described previously (Bird 1995). Soil samples taken adjacent to the river using a 4.7 cm diameter corer were treated in a misting machine as described by Yeates & Bird (1994). Samples of water-saturated soil at the rivers' edges were also collected using the corer but this soil was mixed with water and sieved through a range of sieves as described by Bird (1999). The 1993 samples were collected throughout the island by the author assisted by H. R. B. Jack and the 1997 samples from Rocky River by A. McK. McHugh assisted by M. McHugh.

Soil from Kyeema Conservation Park, supplied by T. Reay and containing *Hemicriconemoides minor*, was also placed in the misting machine and the living nematodes extracted and photographed.

Treatment of nematodes

Living nematodes under a dissecting microscope were picked from the containers into which they had

² Playford Rd Mitcham S. Aust. 5062.

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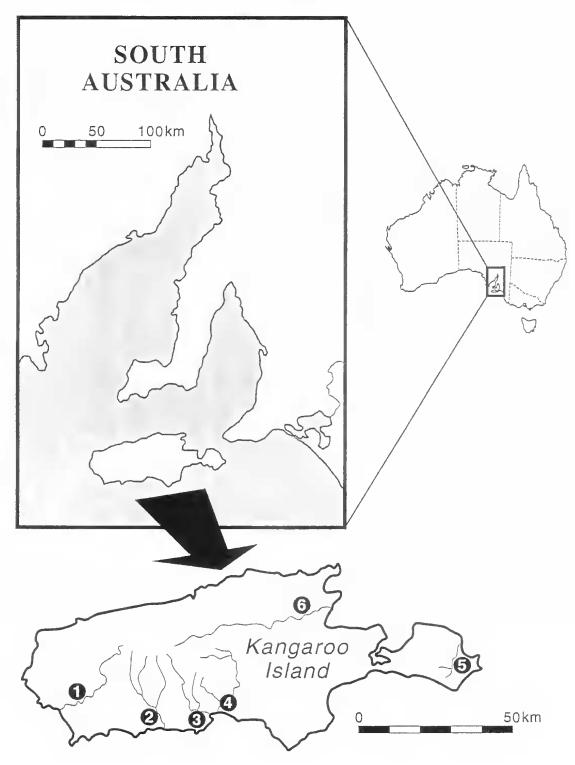


Fig. 1. Map showing collecting sites with rivers listed in order of increasing salinity (see Table 1). (1) = Rocky. (2) = Stunsail Boom. (3) = Harriet. (4) = Eleanor. (5) = Chapman. (6) = Cygnet.

been separated and fixed in hot FA 4:1 before being processed to anhydrous glycerol as described previously (Bird 1995). Both living and fixed nematodes were photographed using a Vanox AHBT research microscope equipped with bright field and interference contrast (Nomarski) optics with Hford Delta 400 film.

The type series has been deposited in the South Australian Museum, Adelaide (SAMA), CSIRO Division of Entomology, Canberra ACT (ANIC) and the Waite Institute Nematode Collection, University of Adelaide (WINC)

De Man's indices and abbreviations for morphological terminology are as follows.

a: body length = maximum body diameter; b: body length + pharyngeal length; e: body length + tail length; c': tail length + body diameter at cloaca; L: total body length; in: length of conus (anterior) part of buccal styler's 100 + total style(length; n: number of specimens; R: number of body annules; RB: breadth of one body unnule; Ran: number of aimules on tail: Rev. munber of annules between labial disc and first annule after secretory-exerctory (S-E) pore; Robaryax west number of annules between labral disc and pharyngo-intestinal valve: Rst: number of annules between labial disc and base of stylet knobs: RV: number of annules from vulva to tail tip; R_{van}: number of annules between vulva and anus; V%: distance of vulva from anterior end x 100 ÷ L; VL; distance between vulva and tail tip: VI-/VB : distance between vulva and tail tip + body width at vulva.

Results

The water environment

Most of the water samples were collected in midwinter when all the springs and rivers had running water. Nevertheless, some of the rivers, such as the Cygnet and Chapman (Fig. 1, Table 1), are clearly estuarine some distance from their mouths. They also have more calcium, magnesium, phosphorus and sulphur than the other rivers listed, particularly Rocky River which runs throughout its length in the Flinders Chase National Park and so is not exposed to agricultural effluents. It is pleasing to note (Table 1) that over the four-year period from 1993-1997 there was no increase in the fonic components in its water; in fact, there appears to have been a slight decrease, possibly due to the difference in the time of year.

Nemanodes

Hemicycliophora fluvialis sp. nov: (FIGS 2-5)

Type: Holotype № Rocky River, K1 (35° 57° S, 136° 42° E), coll. A. F. Bird. 3.vi.1993, SAMA AHC 28115.

Paratypes: 10 ∓ ₹, same data as holotype, SAMA AHC 28115, ANIC 700, WINC 2022.

Description

Body straight to ventrally curved, outer curicle loose fitting. Outer cuticle with circumferential surface markings on either side of narrow band or groove running unbroken through centre of each annule. No breaks observed in annulations. No lateral lines apparent. Lip region continuous with body annules. Labial disc distinct and curved. Three lipannules, the third being largest. Stylet long, basal knobs posteriorly sloped and rounded with posterior eavity. Median bulb, isthmus and terminal bulb of pharynx distinct. Secretory-exerctory (S-E) pore at junction of pharynx and intestine or slightly anterior. Genital branch single, outstretched. Spermatheca oval, containing sperm in all specimens examined. Vulval lips irregular. Post-vulval region cylindrical. tapering towards tail terminus annulated to its tip. Anus obscure and not observed.

 $\begin{array}{l} \textit{Female} \; (\text{Measurements of holotype}) \; (\text{Figs 2-5}) \\ \text{Length 1109 } \mu m; \; a = 32; \; b = 5.7; \; V = 86; \; VL = 136 \\ \mu m; \; VL/VB = 4.3; \; \text{stylet 116 } \mu m; \; m = 86; \; R = 351; \\ R_{ex} = 53; \; RV = 50; \; R_{st} = 31; \; R_{pharynx \; (oes)} = 55. \end{array}$

TABLE 1: Analyses of major soluble ions (mg 14) in water from various rivers on Kangaroo Island.

Date	River	Na	C1	Ca	Mg	K	P	S	* EC	TSS
3 June 1993	Stunsail Boom	269	4.38	12	30	5.5	<0.3	12	1.3	().()9
-0.4-	Harriet	1940	3160	68	181	59	().3	118	1).(1	(1.64
16.0	Eleanor	1930	3370	167	205	27	0.3	79	9.8	0.65
1943	Chapman	3610	4430	460	.586	82	0.5	334	17.8	1.19
18	Cygnet	4750	6970	540	650	37	1.0	257	22.4	1.50
***	Rocky	87	148	2.8	11	2.5	<0.3	4.5	0.45	0.03
October 1995	7 Rocky	62,6	III	2.5	7.7	2.5	0.1	3.0	0.45	0.03

EC = electrical conductivity (deci-siemens m*).

^{**} TSS = total soluble salts (estimated percentage).

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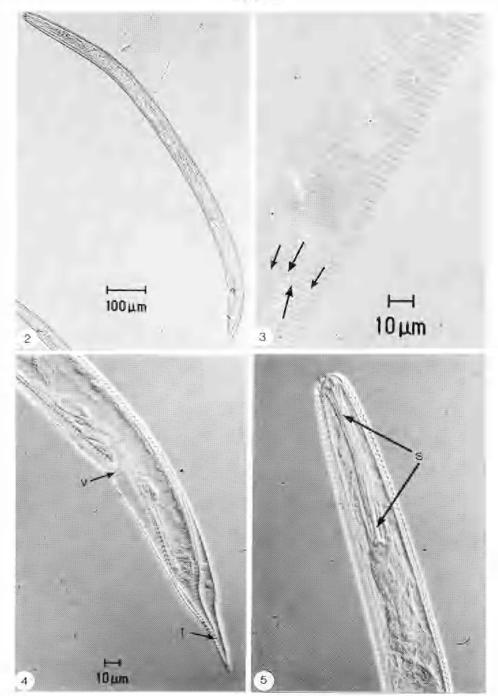


Fig. 2. Hemicycliophora fluvialis sp. nov. Holotype female, showing dimensions of the whole nematode.

Fig. 3. Surface of outer enticle of holotype, showing narrow band or ridge running unbroken through the centre of each annule from side to side (small arrows). The annules also run unbroken across the surface of the cuticle (large arrows) and there is no evidence of lateral lines.

Fig. 4. Tail region of the holotype at higher magnification showing vulva (v) and annulated tapering tail. Note the shorter distance between vulva and tail (i) contrasted with that of H. charlestoni (Fig. 6).

Fig. 5. (Same magnification as Fig. 4). Head of holotype showing the long stylet (s) with its posteriorly sloped basal knobs, the distinct median bulb, isthmus and terminal bulb. Paratype females (Measurements Table 2)

Etymology

The name is derived from L. fluvialis, of or belonging to a river.

Diagnosis and relationships

Hemicycliophora fluvialis sp. nov. resembles H. charlestoni Reay, 1984 but differs in having its vulva closer to the tail tip, fewer annules between its vulva and tail tip, no observable lateral lines, unbroken mid-annular transverse bands or grooves and a lower VL/VB ratio (Figs 3, 4, 6, Table 2). Hemicycliophora fluvialis differs from H. litoralis Reay, 1984 in having a shorter distance between its vulva and tail tip, no observable lateral lines, unbroken mid-annular transverse bands or grooves, fewer annules between its S-E pore and the tip of its head, a lower VL/VB ratio and in the absence of the characteristic vulval fold of the outer cuticle found in most H. litoralis (Figs 3, 4, 7, Table 2) (Reay 1984; Ye & Geraert 1997). The new species differs from H. wallacei

Reay, 1984 in having a much larger stylet (114 μm compared with 82 μm), more annules between its vulva and tail tip and a higher VL/VB ratio (Table 2) and from *H. eucalypti* Reay, 1984 in having a lower De Man's index b, a larger stylet (114 μm compared with 104 μm), more annules, a higher R_{ex} , RV and VL/VB ratio (Table 2).

Eutobrilus heptapapillatus (Joubert & Heyns, 1979) Tsalolikhin, 1981 (FIGS 8-10, Table 3)

Material examined

7 & & Rocky River, K1 (35° 57' S, 136° 42' E) coll. A. F. Bird, 3. vi. 1993, SAMA AHC 28116, ANIC 701, WINC 2023.

Measurements: Table 3

Relationships and remarks

Eutobrilus heptapapillatus is one of the most common nematode inhabitants at the water's edge of

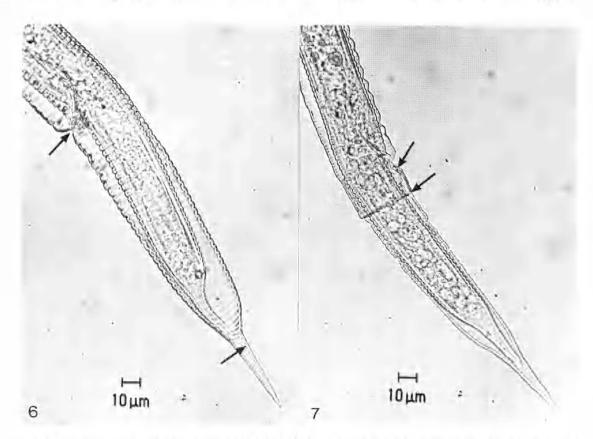


Fig. 6. Tail region of H. charlestoni (paratype ♥WINC 168A - (K)) showing the distance between sulva and tail up (arrows) for comparison with that of H. fluvialis sp. nov. (Fig. 4).

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Table 2. Comparisons of measurements of females of Hemicycliophora fluvialis sp. nov. from Rocky River (KI) with those published for other species from South Australia.

		fluvialis n = 10	H. chara (Reay n =	1984)	H. lite (Reay n= .	1984)	H. wall (Reay 1) n = 1	1984)	H. euce (Reay 1 n =	1984)
Parts measured (µm)	Range	Mean SD	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Body length (L)	974-1278	1096 ±83	1000-1420	1222	850-1380	1114	870-1130	1007	870-1200	1056
De Man's index a	29-39	34 ±4	*¹nd		nd		nd		nd	
р	5,2-5,9	5.5 ± 0.3	5.3-6.5	6.0	4.7-6.6	5.5	5.1-6.3	5.9	5.4-6.7	6.2
Vº/c	85-88	86.2 ±1.0	82-87	84	82-86	84	87-90	89	86-89	87
VL	120-160	138 ±13	159-220	195	156-200	178	101-131	111	116-148	137
Stylet length	107-118	114 ±4.5	100-120	112	94-118	109	77-88	82	97-113	104
m	79-88	82 ±3.2	82-84	83	82-85	84	79-85	83	83-87	84
K	279-352	307 ±27	277-316	297	299-380	326	267-305	285	190-221	206
Rex	50-54	52 ±1.5	49-58	53	60-73	66	49-57	53	39-44	41
R _{SI}	25-33	30 ± 2.6	nd		nd		nd		nd	
R _{pharynx (oes)}	48-58	52 ±3.4	nd		nd		nd		nd	
RV	49-59	53 ±3.2	54-65	60	48-69	55	31-44	36	30-37	33
VL/VB	4.0-4.8	4.4 ± 0.3	4.5-6.3	5.2	4.8-7.3	5.7	3.1-3.9	3.5	3.2-4.2	3.7

^{*}ind = not determined.

Table 3. Comparisons of measurements of males of Eutobrilus heptapapillatus from Rocky River (KI) with other populations.

		7 River = 7	South Africa (Swart & Heyns 1988) n = 7		Lake Albert (Bird 1995) n = 5		Lake Alexandrina (Bird 1995) n = 5	
Parts measured (µm)	Range	Mean SD	Rangé	Mean	Range	Mean	Range	Mean
Body length (L)	1700-2136	1952 ± 185	1550-2120	1920	1873-2000	1931	1800-1990	1896
Max, body width	45-68	55 ± 7	≈Ind	53	64-77	71	60-70	66
Pharynx (oes) length	303-361	331 ± 24	nd	369	305-327	311	270-315	290
Tail length	187-209	199 ± 7	211-300	244	168-191	179	140-192	173
Body width at anus	30-34	33 ± 1	nd	38	36-41	38	32-40	38
Spicule	50-57	54 ± 3	48-57	53	50-55	54	52-56	53
Gubernacutum	27-33	30 ± 2	35-39	37	23-36	31	30-36	33
De Man's index a	31-40	36 ± 3	32-41	.36	26-30	27	28-31	29
ь	5,2-6.2	5.9 ± 0.3	5.1-5.3	5.2	5.7-6.6	6.2	6,1-7.0	6.6
·· · · · ·	8,5-10.5	9.8 ± 1	6.2-8.8	7.9	10,4-11.6	10.8	9,9-12.9	H1.1
F	5.7-6.7	6.1 ± 0.3	5.8-8.0	6,5	4.4- 5.3	4.7	4.4-4.8	4.6
*77 dist. S5-S4	19-26	22 ± 2	16-18	17.4	16-23	20	17-22	19

^{*1} nd = not determined.

^{*19.} dist. S_5 - S_4 = distance between supplementary organs 5 and 4, expressed as a percentage of the sum of the distances between these supplements (Bird 1995).

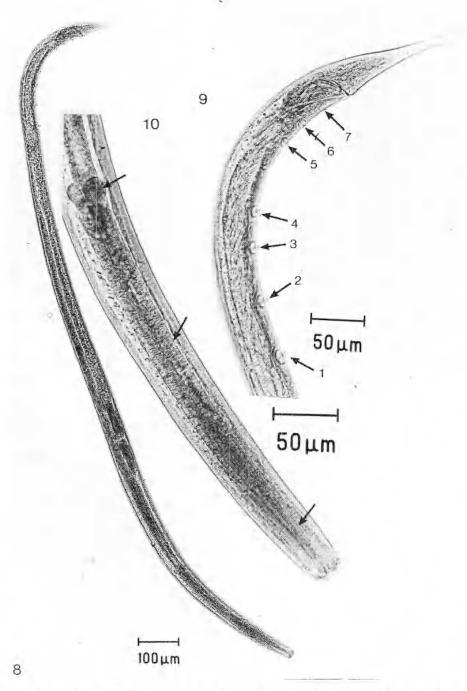


Fig. 8, Montage of whole Eutobrilus heptapapillatus ♂ showing its overall thinner appearance than the same species from the mainland lakes (Table 3).

Fig. 9. Tail region of nematode shown in Fig. 8 at a higher magnification and showing the supplementary organs (numbered arrows).

Fig. 10. Head region of nematode shown in Fig. 8 at a higher magnification and showing the pharynx and associated glands (arrows).

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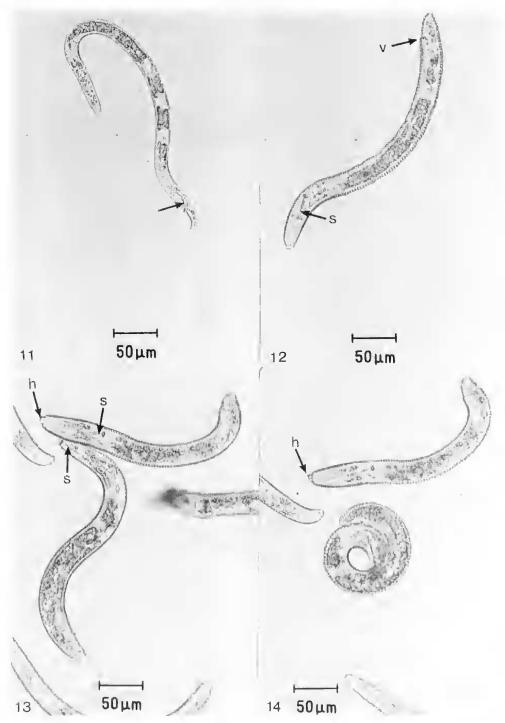


Fig. 11. Living Hemicriconemoides minor

β showing shape and size. Note copulatory spicules (arrow) and absence of a buccal stylet.

Fig. 12. Living H. minor ♀ showing shape and size. Note position of vulya (v) and the pronounced buccal stylet (s).
Figs 13 & 14. Living H. minor ♀ showing evidence for serpentine movement (Fig. 13) and ring formation (Fig. 14). Note the off set heads (h) (cephalic annules) and the buccal stylets (s). These mainland specimens have identical measurements to the Rocky River population (Table 4).

Rocky River, making up almost 40% of the nematode population of the samples collected. This E. heptapapillatus population appears to be morphologically intermediate between the South African population (Swart & Heyns 1988) and those from Lakes Albert and Alexandrina in South Australia (Bird 1995). The population from Kangaroo Island resembles its South African counterpart in maximum body width and De Man's indices a and c' and is thinner than the populations from the South Australian lakes. (Fig. 8, Table 3). It resembles the lake populations in the size of the gubernaculum and percentage distance between the supplementary organs S5 and S4 (Fig. 9, Table 3). The Kangaroo Island population is intermediate between the South African and South Australian lakes populations in pharynx length (Fig. 10, Table 3), tail length and De Man's indices b and e. It has a narrower body width at the level of its anus than any of the other populations but all the populations resemble each other in body length and spicule size (Table 3).

The morphological differences between the Rocky River population of *E. heptapapillatus* and populations of this species from Lakes Alexandrina and Albert may be a reflection of the isolation of Kangaroo Island from the mainland of South Australia some 9,500 years ago (Lampert 1979). It is

thought that prior to separation from the mainland the ancient River Murray ran past the eastern tip of Kangaroo Island less than 10 km away from it. The subsequent retreating of the river, the formation of the island and the onset of more arid conditions, as indicated by changes in the vegetation, would have subjected the tobrilids in Rocky River to environmental pressure greater than those in the billabongs of the River Murray.

Hemicriconemoides minor Brzeski & Reay, 1982 (FIGS 11-14, Table 4)

Material examined

15 ♀ ♀ from soil adjacent to Rocky River, KI (35° 57° S, 136° 42° E) coll. A. E. Bird, 3. vi. 1993, SAMA AHC 28117, ANIC 702, WINC 2024.

Remarks

Kuitpo Forest near the township of Meadows and 30 km south of Adelaide is the type locality for *Hemicriconemoides minor*. However, this species is widely spread throughout the southern parts of Australia and has been found in virgin karri and marri forests south of Manjimup, Western Australia, in forest soil near Cape Jervis, South Australia, in

TABLE 4. Comparisons of measurements of females of a Hemicriconemoides minor population collected close to the banks of Rocky River (35° 57' S, 136° 42' E) on Kangaroo Island compared with those of the paratypes and holotype from a Kuitpo Forest population on the mainland of South Australia.

		Ro	cky River		Kuitpo Forest (Brzeski & Reay 1982)				
Parts measured (µm)			n = 15		Paraty n =	Holotype			
		Range	Mean	SD	Range	Mean			
Body length	(L)	293-383	328.8	± 21.4	290-370	320	340		
De Man's in	dex a	13.6-17.7	15.4	± 1.3	12-15	14	15		
~	b	3.0-3.9	3.3	± 0.3	2.8-4.4	3.4	3.3		
140	c	nd.	n	d	19-27	2.3	26		
-	ν	87.7-94.9	91.1	± 2. F	91-94	92	92		
VL/VB		1.2-1.5	1.3	± 0.1	1.2-1.5	1.3	1,5		
Stylet		63.3-70.0	65.3	± 2.1	56-68	63	65		
R		110-127	118	± 4.5	112-125	118	117		
RB (n=5)		3.3-4.1	3.5	± 0.4	nd	nd	nd		
R _{SI}		24-26	25.1	± ():5	nd	nd	nd		
R _{pharyns} (o	es)	36-41	38.5	± 1.5	nd	nd	nd		
RV		9-11	10.1	± 0.6	10-13	12	13		
Rev		nd	n	ď	32-39	37	36		
R _{van}		nd	n	d	2-5	4	-5		
Ran		nd	n	d	6-8	7	7.		

[&]quot;nd = not determined.

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woodland adjacent to the River Murray in the Sunraysia district of Victoria, on the slopes of Mt William in the Grampian Mountains of Victoria and in rainforest near the Hellger River, 64 km south of Burnie in Tasmania (Brzeski & Reay 1982: Reay & Colbran 1986). It is thus not surprising that it has now been found on Kangaroo Island in the soil under native bush in Flinders Chase National Park about 20 m from Rocky River.

Comparison of measurements of the Flinders Chase females with the holotype female and paratype females of *H. minor* from Kuitpo Forest (Table 4) show that they are remarkably similar.

Hemicriconemoides minor belongs to the family Criconematidae. As its specific name suggests, is a small nematode with the adult female having a characteristic stubby shape (Figs 12-14). Both males and females are about 1/3 mm in length. Criconemands are commonly known as ring nematodes because of their sausage shaped body that may bend into a ring in the living state (Fig. 14). These nematodes have pronounced body annules and a long stylet. The genus Hemiericonemoides is characterized by the temale having a double cutiefe, the outer being sheath-like with retrose annulations. The head may be rounded in outline (Fig. 12) or offset, as can be seen in the living state (Figs 13, 14). The spermatheca in the H. minor specimen depicted in Figure 12 is filled with spenn and the vulva is open. Males were not found in soil from the sample site adjacent to Rocky River although they have been described from the Grampian Mountains in Victoria (Reay & Colbran 1986) and were found in soil from Kyeema Conservation Park, east of Williamsa and south of Adelaide (Fig. 11). They are narrower than females and lack a buccal stylet. Males have not been found in many of the sites from which females have been described.

Because if was not possible to locate clearly the positions of either the S-E pore or the anus in the 15 female specimens measured from Rocky River (Table 4), figures for De Man's index c and R_{ex}-R_{van} and R_{on} are not given. However, figures for RB, R_{st} and R_{pharvax (oes)}, which have not previously been determined, are provided. In all cases where comparable measurements have been made (Table 4) the Rocky River population closely resembles the 16 paratype females of *H. minor* from Kuitpo Forest, in spite of a physical separation by sea for 9.500 years.

Discussion

It is interesting to speculate upon the effects of environmental change on animal populations. Both H. fluvialis and E. heptapapillatus collected from

water logged soils at the water's edge of Rocky River showed differences from closely related or similar species/populations on the mainland of South Australia whereas the population of H. minur collected from soil adjacent to the river but under natural vegetation was indistinguishable from a species/population on the mainland (Table 4). It is likely that the environment of the river bed in Rocky River which dries up into pools in the summer and the site up river from the road bridge which dries out completely (D. Smitherson pers. comm. 1999) would fluctuate much more than that of the bottoms of lakes Albert and Alexandrina where other populations of E. heprapapillams are found. Subjection to regular stresses of drying and wetting could explain why, for instance. E. heptapapillarus from Rocky River may have some similar morphological characteristics to E. hepropapillaries from a stagnant water hole in the Tsitsikama National Park in Cape Province, RSA, which neither population shares with those of E, heprapapillatus from the two lakes (Table 3). An example of this can be found in the maximum widths. That of E. heptapapillatus from the lakes is greater than that of the specimens from Rocky River and Tsitsikama National Park (71 and 66 µm compared with 55 and 53 µm). It would be interesting to know if the Kangaroo Island and South African populations have greater capability of surviving desicention than the lakes populations.

Environmental fluctuations at the site where *H. minor* was collected, under native vegetation in soil some 20 in from the river's edge, would not be nearly as great as at the riparian site and would be similar to the various mainland sites where *H. minor* has been collected. This may account for their close morphological similarities (Table 4).

Although a considerable amount of research has been done on the macrofauna of Kangaroo Island by many workers (Tyler et al. 1979) there has been little or no research into microscopic soil and fresh water nematodes. However, they are very much a part of the soil and water environment and are a natural component of any studies on environmental biology and biodiversity.

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